PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/GB2004/005435	International filing date *(day/month/year)* 23.12.2004
Priority date *(day/month/year)* 23.12.2003	
International Patent Classification (IPC) or both national classification and IPC C12N15/81, C12N1/19	
Applicant DELTA BIOTECHNOLOGY LIMITED	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2004/005435

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-63
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-63
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-63
	No:	Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. By defining a site in the 2µm-family of plasmid that allows to insert heterologous DNA without creating an unacceptable loss of multicopy stability of the plasmid, the present inventors have overcome the prejudice that the 2µm-family plasmids cannot be suitably used as expression vectors. The subject-matter of the present invention is therefore regarded as novel and to involve an inventive step as required by Article 33 PCT.

2. Claim 40 concerns a host cell in which the stability of the marker is at least 1% after 5 generations. It appears that a loss of 99% of the plasmid is in fact an unacceptable loss of the plasmid and hence, the subject-matter of claim 40 as presently formulated cannot be regarded to involve an inventive step and violates Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

1. The claims concern a 2µm-family plasmid comprising a polynucleotide sequence insertion, deletion and/or substitution between the *REP2* or *FLP* gene and the FRT site. However, the application as filed only concerns insertions into this region and there is no support for deletion and/or substitutions and therefore these terms should be deleted as in its present form the claim is not supported by the description as required by Article 6 PCT.

1.1 The same remark is made with respect to claim 34 (c) and (d).

2. The invention concerns yeast expression vectors based on the 2µm-family plasmid, wherein the insertion site for the sequence to be expressed is chosen such that the resulting plasmid is still maintained in a stable manner. However, the subject-matter of claims 48 and 55 concerns **any** yeast vector as long as it has a selectable marker allowing the host cell to survive that cannot be complemented by modifications to the culture medium. However, the essential feature that it has to be a member of the 2µm-family of plasmids and that the marker gene is inserted in the region specified in claim 1 is missing. This is regarded as an essential feature of the invention that has to be mentioned in the

claims. This objection will be met by making claim 48 dependent on claim1.

International Preliminary Examining Authority
European Patent Office
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20 October 2005

Dear Sirs

International Patent Application No PCT/GB2004/005435
DELTA BIOTECHNOLOGY LIMITED
Our Ref: DELBE/P32301PC

This is a response to the Written Opinion of the International Searching Authority (the "ISA") dated 21 April 2005.

Inventive step of Claim 40

In item V of the Written Opinion, the ISA alleged that, insofar as Claim 40 encompasses a host cell in which plasmid stability can be as little as 1% after 5 generations, it lacks an inventive step. With respect, we disagree.

The present application provides 2μm-family plasmids with insertions, deletions and/or substitutions which are useful, or can be modified to be useful, in cell culture processes in which a selective pressure is employed to maintain plasmid presence. Common selectable markers include a β-lactamase gene for ampicillin resistance and a LEU2 marker gene for use in a host cell that has no chromosomal copy of the LEU2 gene (for example, see page 4, lines 15-16 of the present application).

Take for example, plasmids comprising the LEU2 selectable marker gene. When a host cell that has no functional chromosomal copy of the LEU2 gene is transformed with a plasmid that includes the LEU2 gene as a marker, and the culture conditions are adapted so as to exclude the amino acid leucine (i.e. selective conditions), then only host cells that contain the LEU2 plasmid will be able to make leucine *de novo* and hence grow and survive. Thus, under selective conditions (i.e. in the absence of leucine in the culture medium) all cells sampled will have the plasmid; i.e. the plasmid will appear to be 100% stable. However, if

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grown in the presence of leucine (i.e. in non-selective conditions) then host cells will not be forced to maintain the plasmid and so *less than* 100% of sampled cells would contain the plasmid. The percentage of cells that contain the plasmid under non-selective conditions depends on the stability of the plasmid under those non-selective conditions.

In other words, one can only really measure plasmid stability in *non-selective* conditions because, under selective conditions, all plasmids appear to be 100% stable.

Even though plasmids may appear to be 100% stable in selective conditions, those plasmids with very low levels of stability are less preferred than those with higher levels of stability, because higher stability leads to a reduced rate of cell death in those cells whose plasmids are lost. Nevertheless, plasmids with ostensibly low levels of stability under non-selective conditions (eg. 1%) can still be commercially useful when used under selective conditions.

The application discloses, at page 14, line 31 to page 15, line 2, that a level of plasmid stability which may appear to be unacceptable when grown in non-selective media can still be beneficial when grown in selective media. For example, a plasmid which uses one of the sites identified by the present invention to integrate a transgene could be as little as 1% stable under non-selective conditions, but this would be a better level of stability under non-selective conditions than if the transgene was integrated elsewhere in the plasmid and, when grown under selective conditions, the plasmid of the present invention could provide a commercially useful benefit compared to an equivalent prior art plasmid that does not use one of the invention's insertion sites.

pDB2711 is an example of such a plasmid, whose stability is only 10% when determined under non-selective conditions according to Example 1, but which provides for a useful enhancement of recombinant protein expression in shake flask culture under selective growth conditions (page 15, lines 2-5).

Accordingly, it follows that plasmids of the invention that appear to show only low levels of stability under non-selective conditions can provide a commercially useful inventive technical benefit over the prior art. Therefore, we submit that Claim 40 is inventive.

Support

In Point 1 of Section VIII, the ISA alleged that the claims do not meet the requirements for support as required by article 6 PCT insofar as they relate to

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2μm-family plasmids having a deletion and/or substitution within a defined region, based on the belief that the examples only concern the insertion of polynucleotide sequences into the defined regions.

With respect, we disagree. Whilst we appreciate that the application is in the PCT International Phase, useful guidance as to the extent to which a claim must be supported by the description can be obtained from the Guidelines for Examination in the EPO, C-III, 6.2 which states that:

> *"A fair statement of claim is one which is not so broad that it goes beyond the invention nor yet so narrow as to deprive the applicant of a just reward for the disclosure of his invention. The applicant should be allowed to cover all obvious modifications of...that which he has described. In particular, if it is* ***reasonable to predict that all the variants covered by the claims have the properties*** *or uses the applicant ascribes to them in the description, he should be allowed to draw his claims accordingly* (emphasis added)."

We submit that, if the disruption of the sequence of a 2μm-family plasmid within the regions defined by the claims by a DNA insertion does not cause an unacceptable reduction in plasmid stability (which the applicant has demonstrated in the examples of this application) then it is perfectly *reasonable to predict* that disruption within the same region(s) by a deletion or substitution will similarly avoid a reduction in plasmid stability. Irrespective of whether the modification is insertion, deletion or substitution, the original nucleotide sequence of the plasmid is disrupted. Therefore one would reasonably predict that these modifications would have similar effects on plasmid stability.

Furthermore, we note that the ISA have failed to provide technical support for the allegation that the claims should limited to insertions. We respectfully refer the ISA to the EPO's Guidelines, C-III, 6.3, which states that –

> *"As a general rule, a claim should be regarded as supported by the description* ***unless there are well-founded reasons*** *for believing that the skilled person would be unable, on the basis of the information given in the application as filed, to extend the particular teaching of the description to the whole of the field claimed by using routine methods of experimentation or analysis. Support must, however, be of a technical character; vague statements or assertions having no technical content provide no basis.*

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> ***The examiner should raise an objection of lack of support only if he has well-founded reasons.*** *Once the examiner has set out a reasoned case that, for example, a broad claim is not supported over the whole of its breadth, the onus of demonstrating that the claim is fully supported lies with the applicant (see VI, 2.4).* ***Where objection is raised, the reasons should, where possible, be supported specifically by a published document"*** *(emphasis added).*

Thus it is clear that an allegation of lack of support should only be made where there are "*well-founded reasons*", and preferably only if the allegation can "*be supported specifically by a published document*". We respectfully submit that, in the present case, the allegations of lack of support are not well-founded, and certainly have not been supported by a published document, as required by the Guidelines.

We respectfully request that the ISA favourably reconsiders this matter.

In any case, the ISA is incorrect in their assessment that the only plasmid modification disclosed by the description is nucleotide addition. pDB2787, which is listed in Table 4A on page 79, contains a 4 base-pair deletion which truncates the *REP2* gene. The deletion was made by linearising pSAC35 with ApaI, and removing the overhanging nucleotides with T4 DNA polymerase, as described at page 74, lines 1 to 13. A blunt-ended 43 base-pair polynucleotide linker was ligated into the plasmid, to produce pDB2787, as described at page 75, lines 1 to 14. Accordingly, pDB2787 contains a 4 bp deletion and a 43 bp insertion. Claims which relate to a plasmid having a deletion are fully supported by the description. A substitution consists simply of a deletion and an insertion at the same site. Therefore, pDB2787 contains a substitution. Accordingly, the claims that relate to a plasmid having a substitution are also fully supported.

Therefore, the claims, as they relate to nucleotide substitutions and deletions are explicitly supported by the description.

In point 1.1, the ISA referred particularly to Claim 34 part (c) and (d) in alleging lack of support. We submit that the foregoing comments show that Claim 34 is fully supported.

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Conclusions

All claims are novel and inventive and fully supported. We respectfully request that the objections raised in the Written Opinion be favourably reconsidered and we look forward to the issuance of a positive IPRP.

Any amendment is not to be construed as abandonment of subject matter.

Yours faithfully
ERIC POTTER CLARKSON

Richard Bassett

sal/jd